BD MAX™ Cdiff

BD MAX™ Cdiff Assay REF: 443418

For *In Vitro* Diagnostic Use
For use with the BD MAX™ System

2°C – 25°C IVD

INTENDED USE

The BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated *in vitro* diagnostic test for the direct, qualitative detection of the *Clostridium difficile* toxin B gene (*tcdB*) in human liquid or soft stool specimens from patients suspected of having *C. difficile* infection (CDI). The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI.

SUMMARY AND EXPLANATION OF THE PROCEDURE

Clostridium difficile is an anaerobic, gram-positive bacillus that is the leading cause of antibiotic associated diarrhea and pseudomembranous colitis in health care facilities[1-2]. Incidence of CDI has been increasing, and severe cases are becoming more common[3,4,5]. CDI disease symptoms range from mild diarrhea to severe colitis, and even bowel perforation and death. The most common risk factor is exposure to antibiotics[6].

The diagnosis of *C. difficile* infection is based upon clinical signs and symptoms, such as diarrhea, as well as laboratory tests or pathologic finding consistent with toxigenic *C. difficile*. It appears that Toxin B is necessary for the development of CDI[7]. Laboratory diagnosis of toxigenic *C. difficile* includes anaerobic culture followed by detection of toxin by tissue culture cytotoxicity testing [8] or by detection of toxin gene(s) by PCR testing. While culture for toxigenic *C. difficile* followed by tissue culture cytotoxicity is highly sensitive and specific, it is also highly technical, time consuming and has very slow time to result (48 to 96 hours) making this method impractical in the management of patients. Enzyme immunoassay (EIA) used for the detection of toxin A and toxin B and glutamate dehydrogenase (GDH, an enzyme found in all *C. difficile* strains), are currently used in many clinical laboratories because results are available the same day, are easy to perform, and are relatively inexpensive. However, the sensitivity is low, especially for the toxin EIAs, which can lead to missed cases of CDI[8]. PCR methods for the detection of toxin A and/or toxin B have been developed and have demonstrated high sensitivity and specificity as compared to toxigenic culture, cell cytotoxicity and immunoassays[9,10]. Additionally, these tests can be performed in approximately 2 hours. The combination of a highly sensitive and specific assay with rapidly available results may allow for prompt targeted treatment of CDI patients and thus potential improvement in patient outcomes, reduced recovery times, and improved infection control practices.

PRINCIPLES OF THE PROCEDURE

A liquid or soft stool specimen is collected and transported to the laboratory. For testing, a disposable 10µL inoculating loop is dipped into the stool material and the contents dispersed into a BD MAX™ Cdiff Sample Buffer Tube. The Sample Buffer Tube is closed with a septum cap and vortexed. A worklist is created and the Sample Buffer Tube, the BD MAX™ Cdiff unitized reagent strip and the BD MAX™ PCR Cartridge are loaded onto the BD MAX™ System. The BD MAX™ System automates sample preparation including target lysis, DNA extraction and concentration, reagent rehydration, and target nucleic acid amplification and detection using real-time PCR. The BD MAX™ System performs results interpretation automatically. The assay also includes a Sample Processing Control (SPC) that is present in the Extraction Tube. The SPC monitors DNA extraction steps, thermal cycling steps, reagent integrity and the presence of inhibitory substances.

Following enzymatic cell lysis, the released nucleic acids are captured on magnetic beads. The beads, with the bound nucleic acids, are washed using Wash Buffer and the nucleic acids are eluted by heat in Elution Buffer. Eluted DNA is neutralized using Neutralization Buffer and transferred to a Master Mix to rehydrate PCR reagents. After reconstitution, the BD MAX™ System dispenses a fixed volume of PCR-ready solution containing extracted nucleic acids into the BD MAX™ PCR Cartridge. Microvalves in the BD MAX™ PCR Cartridge are sealed by the system prior to initiating PCR to contain the amplification mixture, thus preventing evaporation and contamination.

The amplified DNA targets are detected using hydrolysis (TaqMan®) probes, labeled at one end with a fluorescent reporter dye (fluorophore), and at the other end, with a quencher moiety. Probes labeled with different fluorophores are used to detect *tcdB* and SPC amplicons in two different optical channels of the BD MAX™ System. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of target DNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'-3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the two optical channels used for the BD MAX™ Cdiff Assay is directly proportional to the quantity of the corresponding probe that is hydrolyzed. The BD MAX™ System monitors these signals at each cycle of the PCR and interprets the data at the end of the program to provide a final result.

REAGENTS AND MATERIALS

REF	Contents	Quantity
443418	**BD MAX™ Cdiff Master Mix** *Dried PCR Master Mix containing tcdB specific molecular probe and primers along with Sample Processing Control-specific molecular probe.*	24 tests
	BD MAX™ Cdiff Strips *Reagent strips containing all liquid reagents and disposable pipette tips necessary for specimen processing and DNA extraction.*	24 tests
	BD MAX™ Cdiff Extraction Tube *Freeze-dried pellet containing DNA magnetic affinity beads, Achromopeptidase and Sample Processing Control*	24 tests
	BD MAX™ Cdiff Sample Buffer Tube	24 tests
	Septum Caps	25

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX™ PCR Cartridges (BD Diagnostic Systems catalog no. 437519)
- VWR Multi-Tube Vortexer (VWR catalog no. 58816-115)
- Vortex Genie 2 (VWR catalog no. 58815-234) or equivalent (to vortex stool specimens only)

- NALGENE® Cryogenic Vial holder (VWR catalog no. 66008-783)
- Disposable inoculating loops (10 µL)
- Disposable gloves, powderless
- Dry, clean containers for the collection of liquid or soft stool specimens
- If culture is performed for External Controls: Pre-reduced agar plate for anaerobes (e.g., Brucella Agar with 5% sheep blood, hemin and vitamin K1 plate, BBL™ catalog no. 297716)

WARNINGS AND PRECAUTIONS

- The BD MAX™ Cdiff Assay is for *In Vitro* Diagnostic use.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Check reagent strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes) (see Figure 1).
- Do not remove desiccant from reagent pouches.
- Do not use reagents if desiccant is not present or is broken inside reagent pouches.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or reuse caps, as contamination may occur and compromise test results.
- Proceed with caution when using chemical solutions as Master Mix and Extraction tube barcode readability may be altered.
- Do not use expired reagents and /or materials.
- To avoid contamination by amplicons, do not break apart the BD MAX™ PCR Cartridges after use. The seals of the BD MAX™ PCR Cartridges are designed to prevent contamination.
- Performing the BD MAX™ Cdiff Assay outside the recommended time ranges can produce invalid results.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- In cases where open-tube PCR tests are conducted in the laboratory, care must be taken to ensure that the BD MAX™ Cdiff Assay, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Gloves must be changed before manipulating reagents and cartridges.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in the CLSI Document M29[11] and in Biosafety in Microbiological and Biomedical Laboratories[12].
- Wear protective clothing and disposable gloves while handling all reagents.
- Wash hands thoroughly after performing the test.
- Do not pipet by mouth.
- Do not smoke, drink, chew or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with local, state, provincial and/or federal regulations.
- Consult the BD MAX™ System User's Manual[13] for additional warnings, precautions and procedures.

STORAGE AND STABILITY

Collected specimens should be kept between 2°C and 25°C during transport. Protect against freezing or exposure to excessive heat.

Specimens can be stored at 2-25°C for a maximum of 48 hours or at 2-8°C for a maximum of 120 hours (5 days) before testing.

BD MAX™ Cdiff Assay components are stable at 2-25°C through the stated expiration date. Do not use expired components.

BD MAX™ Cdiff Master Mix and Extraction Tubes are provided in sealed pouches. To protect product from humidity, immediately re-seal after opening. Reagent tubes are stable for up to 7 days at 2-25°C after initial opening and re-sealing.

INSTRUCTIONS FOR USE

Specimen Collection/Transport

In order to obtain an adequate specimen, the procedure for specimen collection must be followed closely. Using a dry, clean container, liquid or soft stool specimens are collected according to the following procedure:

1. Transfer liquid or soft stool (but not urine) into the container. Avoid mixing toilet paper, water or soap with the sample.
2. Label the container.
3. Ship the container to the laboratory according to hospital standard operating procedures (Refer to "Storage and Stability" section).

Specimen Preparation

Note: One (1) Sample Buffer Tube, one (1) Septum Cap, one (1) Master Mix, one (1) Extraction Tube and one (1) Strip are required for each specimen and each External Control to be tested. Remove the required number of tubes/strips from their protective pouches or boxes. Remove the excess air and close the pouches with the zip seal.

1. Label a Sample Buffer Tube (clear cap) with the appropriate specimen identification making sure not to obscure, write or label over the barcodes on the Sample Buffer Tube.
2. Vortex the specimen at high speed for 15 seconds and dip a 10 µL inoculating loop into the liquid or soft stool for testing. For soft stool specimens, remove any excess stool present on the outside of the loop in order to obtain approximately 10 µL.
3. Remove the cap from the Sample Buffer Tube then place the loop into the liquid. Roll the shaft of the inoculating loop between your fingers in order to release the specimen in the tube.
4. Seal the tube with a Septum Cap.
5. Place the Sample Buffer Tube in a NALGENE® Cryogenic Vial holder.
6. Prepare any additional specimens for testing by repeating Steps 1 through 5, then, proceed immediately to Step 7.
7. Vortex all prepared samples simultaneously at maximum speed for one (1) minute with the Multi-Tube Vortexer. The BD MAX™ Cdiff Assay must be performed immediately after the vortexing step.

BD MAX™ System Operation

Note: Refer to the BD MAX™ System User's Manual for detailed instructions (Operation section).

Note: The BD MAX™ Cdiff Assay must be performed immediately after the vortexing step above ("Specimen Preparation", Step 7).

Note: It is recommended to use the reagent tubes removed from their protective pouch (unreconstituted) within 3 hours.

1. Power the System on (if not already done) and log on by entering **<user name>** and **<password>**.
2. Remove the required number of BD MAX™ Cdiff Reagent Strips from the BD MAX™ Cdiff kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes.
3. Remove the required number of Cdiff Extraction Tube(s) and Cdiff Master Mix tube(s) from their protective pouches. Remove excess air, and close pouches with the zip seal.
4. For each sample to be tested, place one (1) BD MAX™ Cdiff Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially with no open spaces.
5. Snap one (1) BD MAX™ Cdiff Extraction Tube (white foil seal) into Position 1 of each of the BD MAX™ Cdiff Strips as shown in Figure 1.
6. Snap one (1) BD MAX™ Cdiff Master Mix Tube (green foil seal) into Position 2 of each of the BD MAX™ Cdiff Strips as shown in Figure 1.

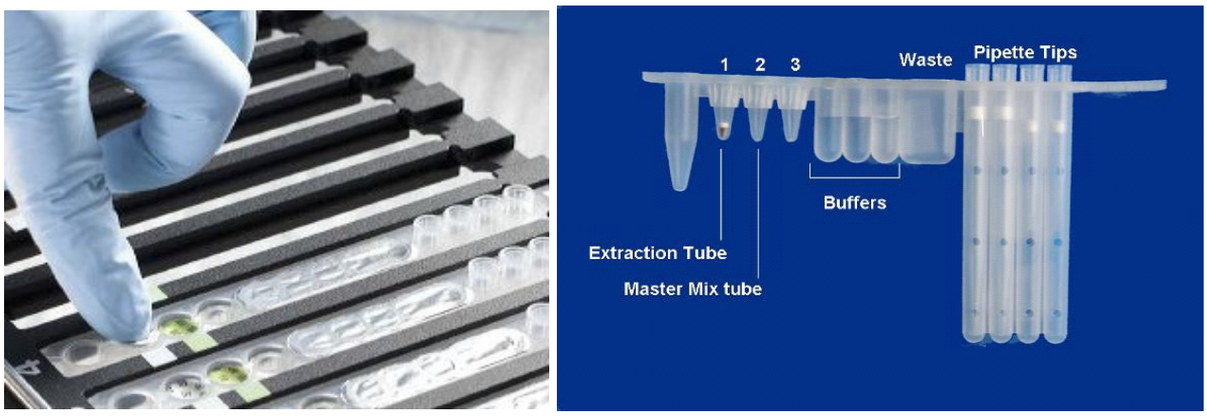

Figure 1: Snap BD MAX™ Cdiff Extraction tubes and Master Mix tubes into reagent strips

7. On the BD MAX™ software, select the **<Consumable info>** tab under the *Run* screen.
8. Enter the kit lot number for the BD MAX™ Cdiff Assay (for lot traceability) by either scanning the 1D barcode with the scanner or by manual entry.
   Note: Repeat Steps 7 and 8 for each new kit lot number.
9. Select the **<Work List>** tab, click on the **<Assay>** field and using the pull down menu, select **<BD MAX Cdiff>**.This will automatically populate the remaining assay fields for Rack A with "BD MAX Cdiff".
10. Enter the BD MAX™ Cdiff Sample Buffer Tube ID, Patient ID and Accession Number (if applicable) for Position 1 of Rack A either by scanning the 1 D barcode with the scanner or by manual entry.
11. Click on the **<Lot Number>** field and using the pull down menu, select the appropriate kit lot number (on the outer box). This will automatically populate the remaining lot number fields for Rack A with the same lot number.
    Note: Step 11 must be repeated for each new kit lot number.
12. Enter the information for Position 2 of Rack A and continue for all remaining Sample Buffer Tubes in the rack.
13. Repeat steps 9 to 12 for Rack B.
14. Place the BD MAX™ Cdiff Sample Buffer Tube(s) in the BD MAX™ Rack(s) following the same order as entered in the worklist.

    Note: Place the tubes into the sample rack with the 1D barcode labels facing outward (this makes scanning tubes easier during sample login).
15. Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 2).
    - Each cartridge accommodates 2 runs of up to 12 samples for a total of 24 samples.
    - The BD MAX™ System will automatically select the position and row on the PCR cartridge for each run.
    - Cartridges are used on a per-run AND rack basis (2 runs per cartridge and 1 cartridge per rack)

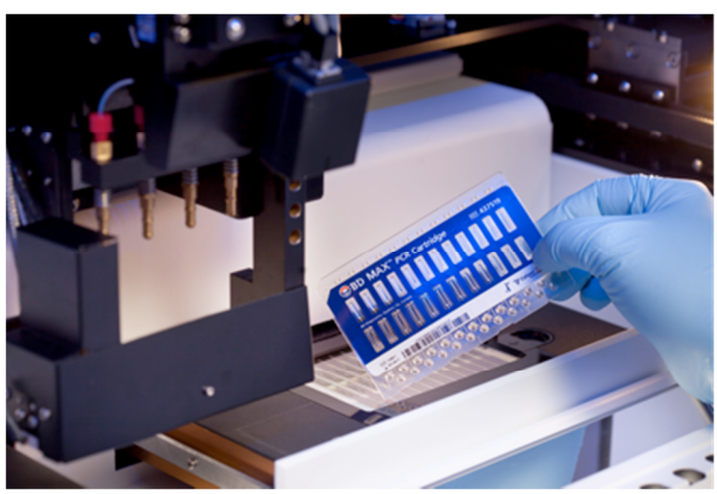

Figure 2: Load PCR Cartridges

16. Load rack(s) onto the BD MAX™ System (Figure 3). Ensure that the placement of rack(s) (left to right) corresponds to the worklist created (top to bottom).

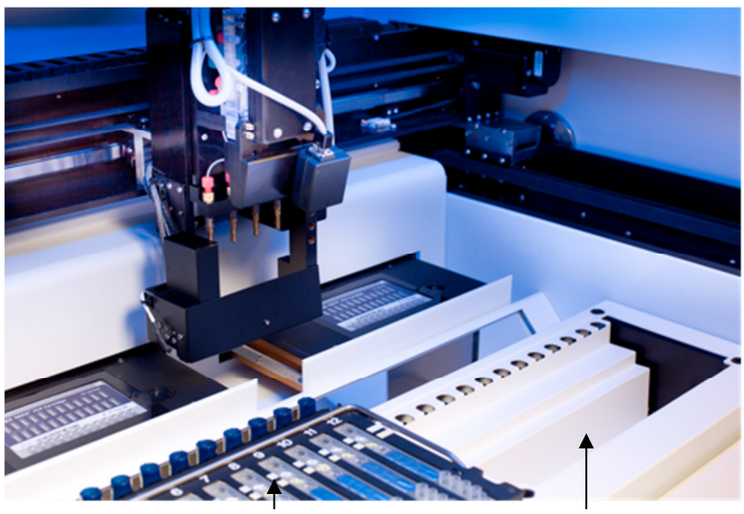

Figure 3: Load Rack(s) into the BD MAX™ System.

17. Close the BD MAX™ System lid and click the **<Start Run>** button to begin processing.
18. At the end of the run check the results immediately, or store the Sample Buffer Tubes at 2-8°C for a maximum of 5 days OR at 25°C for a maximum of 5 hours, until the results are checked.

Note: If a septum cap was damaged during the run, replace it with a new one before storing the specimen.

Note: BD MAX™ Cdiff Sample Buffer Tubes can be stored at 2-8°C for a maximum of 120 hours (5 days) OR at 25°C for a maximum of 5 hours after the end of the run. If an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained, or if an External Control failure occurs, a repeat test from the Sample Buffer Tube must be performed within this timeframe (see "Repeat Test Procedure" section).

QUALITY CONTROL

Quality control procedures monitor the performance of the assay. Laboratories must establish the number, type and frequency of testing control materials according to guidelines or requirements of local, provincial, state and/country regulations or accreditation organizations. For general QC guidance, the user may wish to refer to CLSI MM03[14] and C24[15].

1. The External Positive Control is intended to monitor for substantial reagent failure while the External Negative Control is used to detect reagent or environmental contamination (or carry-over) by *tcdB* amplicons. External Control materials are not provided by BD. Various types of External Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program:
   - Commercially available control materials (e.g., ATCC® 43255, a *C. difficile* strain bearing the *tcdB* gene, and ATCC® 700057, a non-toxigenic *C. difficile* strain, can be used as positive and negative controls, respectively);
   - Suspensions of bacterial strains characterized by the user as toxigenic or non-toxigenic;
   - Previously characterized specimens known to be positive or negative for toxigenic *C. difficile.*

   Note: It is recommended that bacterial strains be freshly prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^7 CFU/mL) from isolated colonies and subsequently diluted with saline to obtain a final concentration of ~3.3 x 10^5 CFU/mL.
2. One (1) External Positive Control and one (1) External Negative Control should be run daily until adequate process validation is achieved on the BD MAX™ System. Reduced frequency of control testing should be based on adequate data and determined by the individual laboratory.
3. An External Negative Control that yields a positive test result is indicative of a specimen handling and/or contamination problem. Review the specimen handling technique to avoid mix-up and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.

4. An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the "System Error Summary" section of the BD MAX™ System User's Manual[13] for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new BD MAX™ Cdiff Assay kit.
5. Each BD MAX™ Cdiff Extraction Tube contains a Sample Processing Control (SPC) which is a plasmid containing a synthetic target DNA sequence. The SPC monitors the efficiency of DNA capture, washing and elution during the sample processing steps, as well as the efficiency of DNA amplification and detection during PCR analysis. If the SPC result fails to meet the acceptance criteria, the result of the specimen will be reported as Unresolved. An Unresolved result is indicative of specimen-associated inhibition or reagent failure. Repeat any specimen reported as Unresolved according to the "Repeat Test Procedure" section below.

RESULTS INTERPRETATION

Results are available on the 'Results' tab in the 'Results' window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets test results. A test result may be called as NEG (negative), POS (positive) or UNR (unresolved) based on the amplification status of the target and of the Sample Processing Control. IND (indeterminate) or INC (incomplete) results are due to BD MAX™ System failure. Results are based on the following decisional algorithm.

ASSAY RESULT REPORTED	INTERPRETATION OF RESULT
POS	*tcdB* gene DNA detected
NEG	No *tcdB* gene DNA detected
UNR	Unresolved – inhibitory specimen or reagent failure
IND	Indeterminate due to BD MAX™ System failure (with Warning or Error Codes*)
INC	Incomplete Run (with Warning or Error Codes*)

* Refer to the "Troubleshooting" section of the BD MAX™ System User's Manual for interpretation of warning and error codes.

REPEAT TEST PROCEDURE

Note 1: Sufficient volume is available for one repeat test from the Sample Buffer Tube on the BD MAX™ System. For Sample Buffer Tubes stored at room temperature, retesting must be performed within 5 hours after the end of the run. Alternatively, for Sample Buffer Tubes stored at 2-8˚C, retesting must be performed within 120 hours (5 days). The remaining stool specimen may also be used for repeat testing within 5 days of collection if stored at 2-8˚C or within 48h if stored at 2-25˚C.
Note 2: New samples may be tested in the same run with repeat samples.

UNRESOLVED RESULT

Unresolved results may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or SPC amplification. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for one (1) minute and restart from the "BD MAX™ System Operation" section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the "Specimen Preparation" section.

INDETERMINATE RESULT

Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for one (1) minute and restart from the "BD MAX™ Operation" section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the

"Specimen Preparation" section. For the interpretation of warning or error code messages, refer to the BD MAX™ User's Manual[13] ("Troubleshooting" section).

INCOMPLETE RESULT

Incomplete results may be obtained in the event that the Sample Preparation or the PCR did not reach its expected time points. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the allowed timeframe defined above. Vortex the sample(s) for one (1) minute and restart from "BD MAX™ Operation" section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the "Specimen Preparation" section. For the interpretation of warning or error code messages, refer to the BD MAX™ User's Manual[13] ("Troubleshooting" section).

EXTERNAL CONTROL FAILURE

External Controls should yield expected results when tested. If specimens have to be repeated due to an incorrect External Control result, the specimens should be repeated from their Sample Buffer Tube along with freshly prepared External Controls within the timeframe defined above. Vortex the samples for one (1) minute and restart from the "BD MAX™ Operation" section. The remaining stool specimen may also be used for repeat testing within the timeframe defined above. Restart from the "Specimen Preparation" section.

CULTURING OF CLINICAL SPECIMENS

To perform species identification directly from stools, clinical specimens may be cultured using hospital procedures.

LIMITATIONS OF THE PROCEDURE

- This product is intended for use only with liquid or soft stools; performance characteristics of other clinical specimen types have not been established.
- This product can only be used on the BD MAX™ System.
- Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the package insert instructions and the BD MAX™ System User's Manual[13] are necessary to avoid erroneous results.
- Good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
- A BD MAX™ Cdiff positive assay result does not necessarily indicate the presence of viable organisms. It does however indicate the presence of the *tcdB* gene and allows for presumptive detection of a *C. difficile* toxigenic organism. The BD MAX™ Cdiff Assay cannot be used for species identification as it does not contain primers and probes specific to *C. difficile.*
- As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection of the assay may be detected, but results may not be reproducible.
- Mesalamine rectal suspension enema and Gynol II® may cause slight inhibition in the BD MAX™ Cdiff Assay (refer to "Interfering Substances" section for further details).
- Tums® and Maalox® liquid may inhibit the BD MAX™ Cdiff Assay (refer to "Interfering Substances" section for further details).
- False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate bacterial cell lysis. The Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Sample Processing Control does not indicate if nucleic acid has been lost due to inadequate collection, transport or storage of specimens, or whether bacterial cells have been adequately lysed.
- BD MAX™ Cdiff Assay results may sometimes be Unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to System failure, and require retesting that can lead to a delay in obtaining final results.

- Mutations or polymorphisms in primer- or probe-binding regions may affect detection of *C. difficile tcdB* gene variants, resulting in a false negative result with the BD MAX™ Cdiff Assay.
- Variant toxigenic *C. difficile* without the *tcdB* gene or with a non-functional Toxin B protein are very rare[16-19]. The BD MAX™ Cdiff Assay targets the *tcdB* gene and it is unknown whether it would detect Toxin A+/Toxin B- variant strains.
- An excess amount of stool may inhibit the BD MAX™ Cdiff Assay.
- As with all *in vitro* diagnostic tests, positive and negative predictive values are highly dependent on prevalence. BD MAX™ Cdiff Assay performance may vary depending on the prevalence and population tested.

EXPECTED VALUES

In the BD MAX™ Cdiff Assay clinical study a total of 1834 reportable results, from specimens compliant at the specimen and PCR levels, were obtained from 6 geographically diverse sites. The study population was grouped into in-patient, out-patient and unknown categories. The number and percentage of positive cases, as determined by the BD MAX™ Cdiff Assay, are presented in the table below:

		BD MAX™ Cdiff Assay		
Group	**Total Number of Specimens[1]**	**Number Positive**	**Number Negative**	**Positive Percentage**
In-patient	**1249**	**184**	**1065**	**14.7% (184/1249)**
Out-patient	**457**	**114**	**343**	**24.9% (114/457)**
Unknown	**128**	**17**	**111**	**13.3% (17/128)**
Total[1]	**1834**	**315**	**1519**	**17.2% (315/1834)**

[1] Total specimens based on compliant PCR results.

PERFORMANCE CHARACTERISTICS

Clinical performance characteristics of the BD MAX™ Cdiff Assay were determined in a multi-site prospective investigational study. Six (6) investigational centers participated in the study. To be enrolled in the study, specimens had to be from patients suspected of having *C. difficile* infection for which diagnostic tests were indicated and ordered. Only soft or liquid stools, and only one specimen per patient, were included.

The Comparative Reference Method consisted of direct culture complemented by enriched culture. Enriched culture analysis was completed for all specimens that were negative for toxigenic *C. difficile* by direct culture. The anaerobic culture was used to isolate *C. difficile,* if present. This was followed by confirmation of the isolate identification and a Tissue Culture Cytotoxicity Assay to determine the toxigenicity of the isolate.

Of the 2071 soft or liquid stool specimens compliant with the Reference Method (Direct and Enriched Culture), 1819 gave compliant and reportable results with the BD MAX ™Cdiff Assay. In comparison to the Reference Method, the BD MAX™ Cdiff Assay identified 87.7% of the toxigenic *C. difficile* positive specimens and 96.8% of the toxigenic *C. difficile* negative specimens (Tables 1 and 2).

Table 1: Results Obtained with the BD MAX™ Cdiff Assay in Comparison to the Reference Method

All Sites		Reference Method		
		+	-	Total
BD MAX™ Cdiff Assay	+	265	48[1]	313
	-	37[1]	1469	1506
	Total	302	1517	1819
Sensitivity: 87.7% (265/302) (95% CI: 83.6%, 91.0%) Specificity: 96.8% (1469/1517) (95% CI: 95.8%, 97.6%) PPV: 83.5% (95% CI: 79.4%, 87.1%) NPV: 97.7% (95% CI: 97.0%, 98.4%)				

[1] Further investigation was performed on specimens with discordant results between the Reference Method and the BD MAX™ Cdiff Assay.

- 27 of 48 False Positive BD MAX™ Cdiff specimens were also found to be positive using another commercially available FDA-cleared RT-PCR assay targeting the *C. difficile tcdB* gene.
- 32 of 37 False Negative BD MAX™ Cdiff specimens, were also found to be negative using another commercially available FDA-cleared RT-PCR assay targeting the *C. difficile tcdB* gene.

Table 2. Results Obtained by Site using the BD MAX™ Cdiff Assay in Comparison to the Reference Method

Site	Sensitivity	Specificity
Site 1	90.0% (18/20) (69.9%, 97.2%)[1]	95.3% (202/212) (91.5%, 97.4%)
Site 2	84.4% (38/45) (71.2%, 92.3%)	95.3% (205/215) (91.7%, 97.5%)
Site 3	94.7% (36/38) (82.7%, 98.5%)	97.9% (275/281) (95.4%, 99%)
Site 4	83.8% (31/37) (68.9%, 92.3%)	95.8% (322/336) (93.1%, 97.5%)
Site 5	96.1% (49/51) (86.8%, 98.9%)	98.4% (186/189) (95.4%, 99.5%)
Site 6	83.8% (93/111) (75.8%, 89.5%)	98.2% (279/284) (95.9%, 99.2%)
Overall Study	87.7% (265/302) (83.6%, 91.0%)	96.8% (1469/1517) (95.8%, 97.6%)

[1]Numbers in parentheses express the 95% confidence interval boundaries

In comparison to direct culture, the BD MAX™ Cdiff Assay identified 96.5% of the toxigenic *C. difficile* positive specimens and 92.7% of the toxigenic *C. difficile* negative specimens (Table 3).

Table 3: Results Obtained with the BD MAX™ Cdiff Assay in Comparison to Direct Culture

All Sites		Direct Culture		
		+	-	Total
BD MAX™ Cdiff Assay	+	194	118	312
	-	7	1507	1514
	Total	201	1625	1826
Positive Percent Agreement: 96.5% (194/201) (95% CI: 93.0%, 98.3%) Negative Percent Agreement: 92.7% (1507/1625) (95% CI: 91.4%, 93.9%)				

Out of 1860 soft or liquid stool specimens tested with the BD MAX™ Cdiff Assay, 58 (3.1%) were initially reported as unresolved. 42 of those were repeated and 32 were resolved upon repeat testing. Overall, 0.5% remained unresolved after repeat (Table 4).

Table 4: Unresolved Rate

Initial Unresolved Rate	Unresolved Rate After Repeat
3.1% (58/1860)* (95% CI: 2.4%, 4.0%)	0.5% (10/1844) (95% CI: 0.3%, 1.0%)

* Total number based on compliant specimens and BD MAX™ Cdiff Assay results.

Out of 1916 soft or liquid stool specimens tested with the BD MAX™ Cdiff Assay, 21 (1.1%) were initially reported as indeterminate. Out of a total of 1844 compliant results, no result remained Indeterminate upon repeat (considering valid results and compliant repeats).

Out of 1916 soft or liquid stool specimens tested with the BD MAX™ Cdiff Assay, 28 (1.5%) were initially reported as incomplete. Out of a total of 1844 compliant results, no result remained Incomplete upon valid repeat (considering valid results and compliant repeats).

Comparison Studies

In addition to the multi-site investigational study, a comparison study was performed using the BD MAX™ Cdiff assay and two (2) commercially available FDA-cleared molecular assays for the detection of the *C. difficile tcdB* gene. Testing was performed on 2013 specimens at two external sites. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the BD MAX™ Cdiff assay, in comparison to the two commercially available FDA-cleared molecular assays, demonstrated excellent overall agreement.

In comparison to one FDA-cleared molecular test (Table 5a), the PPA and NPA of the BD MAX™ Cdiff Assay were 99.1% (CI: 94.9%-99.8%) and 97.4% (CI: 95.7%-98.4%), respectively.

In comparison to a second FDA-cleared molecular test (Table 5b), the PPA and NPA of the BD MAX™ Cdiff Assay were 95.5% (CI: 92.1%-97.5%) and 98.8% (CI: 97.9%-99.3%), respectively.

Table 5a: Results Obtained with the BD MAX™ Cdiff Assay in Comparison to a Commercially Available FDA-cleared Molecular Assay for *C. difficile*.

All Sites		FDA-cleared Molecular Assay 1		
		+	-	Total
BD MAX™ Cdiff Assay	+	106	14	120
	-	1	526	527
	Total	107	540	647
Positive Percent Agreement: 99.1% (95% CI: 94.9%, 99.8%) Negative Percent Agreement: 97.4% (95% CI: 95.7%, 98.4%)				

Table 5b: Results Obtained with the BD MAX™ Cdiff Assay in Comparison to a Second Commercially Available FDA-cleared Molecular Assay for *C. difficile*.

All Sites		FDA-cleared Molecular Assay 2		
		+	-	Total
BD MAX™ Cdiff Assay	+	233	14	247
	-	11	1108	1119
	Total	244	1122	1366
Positive Percent Agreement: 95.5% (95% CI: 92.1%, 97.5%) Negative Percent Agreement: 98.8% (95% CI: 97.9%, 99.3%)				

Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LoD) for the BD MAX™ Cdiff Assay was determined as follows: individual inoculating loops were dipped into a wide range of *C. difficile* bacterial suspensions at different concentrations, prepared and quantified from cultures of 4 *C. difficile* strains representing 3 toxinotypes (0, III, VIII). Each loop was then transferred to a SBT, already containing fecal matrix negative for toxigenic *C. difficile*. Each *C. difficile* strain was tested in replicates of 24 per concentration, by 2 different operators, using 3 different production lots of the BD MAX™ Cdiff Assay. Analytical sensitivity (LoD), defined as the lowest concentration at which 95% of all replicates tested positive, ranged from 125 to 265 CFU per loop (Table 6).

Table 6: Limit of Detection of the BD MAX™ Cdiff Assay

C. difficile Strain	Toxinotype	LoD in CFU per loop
ATCC® 43255	0	265 (95% CI: 140, 502)
ATCC® 9689	0	156 (95% CI: 82, 298)
ATCC® BAA-1805	III	205 (95% CI: 102, 412)
ATCC® 43598	VIII	125 (95% CI: 66, 235)

Analytical Inclusivity

A variety of toxigenic *Clostridium difficile* strains were included in this study taking into account geographic origin, toxinotype, NAP1 outbreaks and temporal diversity. Sixty-four (64) strains including 23 toxinotypes[20-22] and representing 21 countries were tested, including strains from public collections and well-characterized clinical isolates. The assay correctly identified 62 of the 64 toxigenic *C. difficile* strains which were tested at ~3xLoD. Two (2) strains [strains IS25 (toxinotype XII) and R9385 (toxinotype XV)] produced low signal results and were false-negative in 1 out of 5 replicates.

Analytical Specificity

The BD MAX™ Cdiff Assay was performed on samples containing phylogenetically related species *(Clostridium* other than toxigenic *C. difficile*) and other organisms (bacteria, viruses) likely to be found in stool specimens.

- Six (6) out of 6 *C. difficile* strains not bearing the *tcdB* gene, tested at a concentration ≥ 1 X 10^8 CFU/mL, produced negative results with the BD MAX™ Cdiff Assay;
- Thirty (30) out of 30 *Clostridium* strains other than *C. difficile*, including 4 strains of *C. sordellii*, tested at a concentration ≥ 1 X 10^8 CFU/mL, produced negative results with the BD MAX™ Cdiff Assay;
- Ninety-five (95) out of 98 other bacterial strains, including 93 species and subspecies, were tested at a concentration ≥ 1 X 10^8 CFU/mL (or ~ 1 X 10^8 genomic DNA cp/mL or 1 X 10^8 elementary bodies/mL) and produced negative results with the BD MAX™ Cdiff Assay. An investigation was conducted and determined the false-positive results were due to contamination. New suspensions of 3 strains were tested and generated the expected negative results.
- Seven (7) out of 7 viruses, tested at a concentration ≥ 1 X 10^5 PFU/mL, produced negative results with the BD MAX™ Cdiff Assay.

Interfering Substances

Twenty-five (25) biological and chemical substances occasionally used or found in perianal, rectal and/or stool specimens were evaluated for potential interference with the BD MAX™ Cdiff Assay. Two (2) organisms (*E. coli* ATCC 25922 and non-toxigenic *C. difficile* ATCC 700057) were also tested at high loads in order to assess bacterial interference. Toxigenic *C. difficile* negative specimens and toxigenic *C. difficile* positive specimens at 2-3X LoD were tested with the highest amount of each compound likely to be found in the specimens or with interfering organisms (1 X 10^8 CFU/mL of each strain). Potentially interfering substances include calcium carbonate (Tums®) as well as magnesium and aluminum hydroxide (Maalox® liquid). Results demonstrated no reportable interference with any other tested substance except for Mesalamine rectal suspension enema and Gynol II® that both showed slight inhibition (delay of Second Derivative Peak Abscissa) in the BD MAX™ Cdiff Assay, however, expected assay results were still obtained (Table 7).

Table 7: Endogenous and Commercial Exogenous Substances tested with the BD MAX™ Cdiff Assay

Brand Name or Description	Weight or Volume Tested/SBT	Result	Brand Name or Description	Weight or Volume Tested/SBT	Result
Nystatin	10 µL	NI	Pepto Bismol™	10 µL	NI
Hyderm™ Hydrocortisone (cream)	0.0246 g	NI	Ex-Lax®	0.0134 g	NI
Glycerin Suppositories	0.0129 g	NI	Metronidazole	10 µL	NI
Ihle's Paste	0.0388 g	NI	Vancomycin	10 µL	NI
Anusol® Plus	0.0123 g	NI	Polysporin®	0.0240 g	NI
Preparation H® with Bio-Dyne® (cream)	0.0222 g	NI	Naproxen	10 µL	NI
Major Prep® with Phenylephrine	0.0111 g	NI	Tucks® Personal Cleansing Pads	3 mm	NI
Tums®	0.0395 g	I	Triglyceride Mix (C2-C10)	10 µL	NI
Maalox® (liquid)	10 µL	I	Palmitic Acid	25 mg	NI
Mesalamine Rectal Suspension Enema	10 µL	*	Stearic Acid	10 mg	NI
Fleet® Mineral Oil Enema	0.0182 g	NI	Blood	10 µL	NI
Gynol II® Vaginal Contraceptive (with Nonoxynol-9)	0.0262 g	*	Mucus	10 µL	NI
Imodium AD®	0.0062 g	NI	*E. coli* + non-toxigenic *C. difficile*	10 µL	NI

I: Interference with the BD MAX™ Cdiff Assay.
NI: No reportable interference with the BD Cdiff™ Assay.
* Mesalamine rectal suspension enema and Gynol II® (with nonoxynol-9) showed slight inhibition (delay of Second Derivative Peak Abscissa) in the BD MAX™ Cdiff Assay, however, expected assay results were still obtained.

Precision

Within-laboratory precision was evaluated for the BD MAX™ Cdiff Assay at one (1) site. The Precision panel consisted of 5 specimen categories as follows:

- Moderate Positive (MP): 2 – 5 x LoD
- Low Positive (LP): 1- 2 x LoD
- High Negative 1:10 (HN1:10): 10-fold dilution of 1 x LoD
- High Negative 1:100 (HN1:100): 100-fold dilution of 1 x LoD
- True Negative (Neg)

Escherichia coli ATCC 25922 was added to every tube in the panel. *C. difficile* strains were tested in each of the specimen categories with the exception of the negative (Neg) specimen category.

Testing was performed in duplicate, over 12 days, with 2 runs per day, by 2 technologists. Precision study results for Neg, LP and MP samples demonstrated 100% agreement. Precision study results for HN1:100 and HN1:10 demonstrated agreement of 95.8% and 58.3%, respectively.

Reproducibility

The Reproducibility panel consisted of 5 specimen categories as follows:

- Moderate Positive (MP): 2 – 5 x LoD
- Low Positive (LP): 1- 2 x LoD
- High Negative 1:10 (HN1:10): 10-fold dilution of 1 x LoD
- High Negative 1:100 (HN1:100): 100-fold dilution of 1 x LoD
- True Negative (Neg)

Specimens in each category were tested in triplicate, on 5 distinct days, wherein each day 2 panels were tested by 2 technologists, at 3 clinical sites using 1 lot of reagents (Site-to-Site). One of these clinical sites participated in an extended study where 2 additional lots of reagents were tested (Lot-to-Lot). Results are shown for each specimen category.

For Site-to-Site Reproducibility, the overall percent agreement was 100% for MP, LP and Neg categories, with 92.2% and 50.0% negative agreement for HN1:100 and HN1:10 categories, respectively (Table 8).

For Lot-to-Lot Reproducibility, the overall percent agreement was 100% for MP, LP and Neg categories, with 96.7% and 64.4% negative agreement for HN1:100 and HN1:10 categories, respectively (Table 9).

Second Derivative Peak Abscissa (SDPA), an internal criteria used to determine a final assay result, was selected as an additional means of assessing assay reproducibility. Overall mean SDPA values with variance components (SD and %CV) are shown in Tables 8 and 9.

Table 8: Site-To-Site Reproducibility Study Results using One Lot of the BD MAX™ Cdiff Assay

Category	SITE						Overall Percent Agreement		SDPA Values[1]		
	Site 1 Percent Agreement		Site 2 Percent Agreement		Site 3 Percent Agreement				Overall Mean	SD	%CV
Neg[1]	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95% CI: 95.9%, 100.0%)	28.7	0.30	1.1
HN1:100[1,2]	28/30	93.3%	25/30	83.3%	30/30	100.0%	92.2%	(95% CI: 84.8%, 96.2%)	28.8	0.39	1.4
HN1:10[1,2]	17/30	56.7%	8/30	26.7%	20/30	66.7%	50.0%	(95% CI: 39.9%, 60.1%)	28.8	0.35	1.2
LP	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95% CI: 95.9%, 100.0%)	32.5	0.77	2.4
MP	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95% CI: 95.9%, 100.0%)	31.6	0.82	2.6

[1]For the Negative and High Negative categories, SDPA values reported are for the SPC. For other categories, SDPA values reported are for the toxigenic *C. difficile* target.

[2] For the High Negative categories, the expected assay result was deemed to be negative. Therefore, percent agreement was calculated for negative results.

Table 9: Lot-to-Lot Reproducibility Study Results using Three Lots of the BD MAX™ Cdiff Assay

Category	LOT						Overall Percent Agreement		SDPA Values[1]		
	Lot 1 Percent Agreement		Lot 2 Percent Agreement		Lot 3 Percent Agreement				Overall Mean	SD	%CV
Neg[1]	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95% CI: 95.9%, 100.0%)	29.0	0.57	2.0
HN1:100[1,2]	29/30	96.7%	28/30	93.3%	30/30	100.0%	96.7%	(95% CI: 90.7%, 98.9%)	29.0	0.57	2.0
HN1:10[1,2]	17/30	56.7%	21/30	70.0%	20/30	66.7%	64.4%	(95% CI: 54.2%, 73.6%)	29.0	0.58	2.0
LP	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95% CI: 95.9%, 100.0%)	32.8	0.78	2.4
MP	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95% CI: 95.9%, 100.0%)	32.1	0.80	2.5

[1]For the Negative and High Negative categories, SDPA values reported are for the SPC. For other categories, SDPA values reported are for the toxigenic *C. difficile* target.

[2] For the High Negative categories, the expected assay result was deemed to be negative. Therefore, percent agreement was calculated for negative results.

Carryover / Cross-Contamination

A study was conducted to investigate within-run carryover and between-run carryover while processing specimens with high bacterial load of toxigenic *C. difficile* in the BD MAX™ Cdiff assay. A panel made of one high positive member and one negative member was used to prepare numerous samples. A *Clostridium difficile* strain (Tox 0, ATCC 9689) was used for the high positive *C. difficile* panel member (~$3x10^8$ CFU/mL). The negative member did not contain any target analyte. Twelve (12) replicates of the high positive panel member and 12 replicates of the negative panel member were tested in each run by alternating negative and positive samples. Three (3) operators performed 3 consecutive runs for a total of 9 runs of 24 samples. There were no false positive results due to carry-over contamination.

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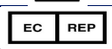	Authorized Representative
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